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How Do Temperature and Salinity Affect Relative Rates of Growth, Morphological Differentiation, and Time to Metamorphic Competence in Larvae of the Marine Gastropod *Crepidula plana*?

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Abstract. The influence of environmental conditions on rates of larval growth has been documented many times for various marine mollusks. But the factors that influence rates of morphological and physiological differentiation, particularly the rate at which larvae within a population become competent to metamorphose, remain obscure. In four experiments, we reared larvae of the gastropod *Crepidula plana* at 29°C, 25°C, and 20°C at 30 ppt salinity, and in two other experiments, in salinities between 4–30 ppt at 25°C. Rates of shell growth and morphological differentiation, and rates of becoming competent within populations were recorded. Larvae were considered to be competent to metamorphose if they could be stimulated to metamorphose by exposure to a high concentration of KCl (20 m*M* above ambient). Larvae consistently became competent faster at higher temperatures, but in only one of four experiments did temperature also consistently increase the rates of growth and morphological differentiation. Larvae took longer to become competent when reared at lower salinities, but the effects were poorly predicted by the influence of salinity on rates of growth and morphological differentiation. Competent larvae could also not be recognized by shell length; many individuals were competent at shell lengths of 600–800 μm, while many other individuals were still not competent at sizes exceeding 1000 μm. At 29°C, many individuals became competent at smaller sizes than those reared at lower temperatures. Presence of gill filaments or shell brims also did not correlate with individual metamorphic competence. The data suggest that growth rate, rate of morphological differentiation, and time required for larvae of *C. plana* to become competent can be uncoupled markedly by shifts in rearing conditions.

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Introduction

Competence is a differentiated state in which larvae of benthic marine invertebrates first become capable of metamorphosing in response to environmental cues (Crisp, 1974; Scheltema, 1974; Chia, 1978; Hadfield, 1978; Miller and Hadfield, 1986; Coon *et al.*, 1990; Fitt *et al.*, 1990). Metamorphosis of gastropod larvae is most easily defined by the loss of the larval velum, an organ responsible for larval feeding, swimming, and gas exchange. This transformation marks the transition from a swimming planktonic stage to a largely sedentary benthic stage. The time required for a larva to become competent thus determines the obligate planktonic dispersal period (Scheltema, 1978; Jackson and Strathmann, 1981).

Larvae are often designated as competent based on their size, age, or the presence of particular morphological characteristics (Bayne, 1964; Bayne, 1965; Bayne, 1971; Hickman and Gruffydd, 1971; Switzer-Dunlap and Hadfield, 1977; Hadfield, 1978; Pechenik, 1984; Lima and Pechenik, 1985; Butman *et al.*, 1988). In at least some molluscan species, however, such criteria may be poor indicators of an individual's competence to metamorphose. In the bivalves *Mytilus edulis* and *Crassostrea gigas*, for example, neither shell size, age, nor the presence of eye spots guarantee that larvae will metamorphose in response to apparently appropriate cues (Eyster and Pechenik, 1987; Coon *et al.*, 1990). Similarly, size is an

inadequate indicator of metamorphic competence for the gastropod *Crepidula fornicata;* larvae from a single larval culture became competent to metamorphose at shell lengths ranging between 700 and 1000 μm (Pechenik and Heyman, 1987, in response to elevated KCl concentrations). Neither did behavioral changes successfully signal the time at which larvae of the opisthobranch *Phestilla sibogae* became metamorphically competent in the experiments of Miller and Hadfield (1986). There is growing reason to doubt, then, that the time required for a larva to become metamorphically competent is directly coupled to the rate at which the larva grows or develops most other conspicuous traits.

To date, few workers have rigorously documented the rate at which larvae in a population become competent to metamorphose, or have considered the influence of environmental factors on that rate. In addition, the correspondence between the rates of larval growth and of attaining metamorphic competence have been poorly explored. Under what conditions do larvae become competent more quickly, and to what extent can this accelerated attainment of competence be predicted from the influence of those conditions on rates of growth or morphological differentiation? Because larval metamorphosis can, for a number of species, be triggered by elevating KCl ambient concentration (Yool *et al.,* 1986; Pechenik and Heyman, 1987), the rate and sizes at which larvae of those species become competent can be determined experimentally. The larvae of *Crepidula fornicata* can be induced to metamorphose by elevated KCl concentrations at about the same age and size that larvae become responsive to adult-conditioned seawater and surfaces bearing microbial films (Pechenik, 1980; Pechenik and Heyman, 1987). The latter probably serve as metamorphic cues in the field (McGee and Target, 1989), but the active constituents have not been isolated.

In this paper, we report the effects of temperature and salinity on the rate of larval growth, the rate of morphological differentiation, and the time required for larvae of the prosobranch gastropod *Crepidula plana* to become metamorphically competent (as indicated by their response to elevated potassium concentration). In *Crepidula plana,* virtually all larvae eventually metamorphose "spontaneously"—no cue is deliberately provided—in glassware that is cleaned and acid-rinsed daily (Lima and Pechenik, 1985). Thus, the *maximum* dispersal potential for these larvae depends on how long metamorphosis can be delayed after they first become competent. We therefore also monitored the timing of "spontaneous" metamorphosis in relationship to the onset of metamorphic competence. We have thus been able to directly determine the influence of temperature on the length of time that metamorphosis can be delayed under laboratory conditions.

Materials and Methods

Maintenance of adults and larvae of Crepidula plana

Adult *Crepidula plana* were collected near Woods Hole, Massachusetts. We maintained adults at room temperature (21–25°C) in 1 μm filtered seawater (collected at Nahant, Massachusetts), changing the seawater daily. We fed adults the green unicellular alga *Dunaliella tertiolecta* (clone *DUN*) daily, until larval release. After their release, the larvae were isolated on a 150 μm sieve and transferred to 0.45 μm filtered seawater (29–30 ppt salinity). In each of the six experiments conducted, the larvae were all released on the same day, but not necessarily from one female.

Larvae were fed the naked flagellate *Isochrysis* sp. (Tahitian strain, clone *T-ISO*) daily; seawater was changed every other day. At the start of an experiment (2–9 days after hatching), known numbers of larvae were randomly assigned to either a 20°C, 25°C, or 29°C temperature incubator (Percival Manufacturing) stable to 0.1°C. Larvae of *C. plana* grow very slowly at temperatures below 20°C, and 29°C seems to be near the upper lethal temperature limit for this species (Lima and Pechenik, 1985). All larvae were cultured on a 11L:13D light cycle. Larval concentrations were maintained below one larva · ml^{-1} in all experiments (I–VI); the aim was to maximize growth rates and minimize competition for food. Larvae were fed 1.8×10^5 cells · ml^{-1} of *T-ISO* every other day in Experiments I and II, and daily in all subsequent experiments. A hemacytometer was used to determine algal cell concentrations. To monitor survival, we removed dead or moribund larvae from the cultures at each water change. Glassware was cleaned with Bon Ami and rinsed with deionized water at each water change.

Determining the influence of temperature on rates of growth and morphological differentiation

In four experiments, we examined how temperature affects the relationship between rates of larval growth, rates of morphological differentiation, and rates of becoming competent to metamorphose. In Experiments I and II, 1100–1600 larvae were reared at each tested temperature (20°C, 25°C, and 29°C) in batch culture. Thirty actively swimming larvae were collected daily (25°C and 29°C), or every other day (20°C) from the batch cultures. Seawater volumes were adjusted after larval collection to maintain larval densities. In Experiments III and IV, we determined the growth rates of larvae reared in individual glass bowls, at densities also below 1 larva · ml^{-1}.

Larval shell lengths were measured at 50× using a dissecting microscope equipped with an ocular micrometer;

Table I

Influence of temperature and salinity on rates of larval shell growth, morphological differentiation and becoming competent for larvae of Crepidula plana

Experiment number	Temperature (°C)	Salinity (ppt)	Growth rate ($\mu m \cdot day^{-1}$)	Days to 50% of the population competent	Days to 50% of the population gilled	Days to 50% of the population brimmed	% Mortality (n)
I	29	30	28.4 ($r^2 = 0.96$)	12.2	—	—	4.0 (1620)
I	25	30	40.0 ($r^2 = 0.96$)	19.0	—	—	7.0 (1120)
I	20	30	33.8 ($r^2 = 0.96$)	23.4	—	—	13.0 (1620)
II	29	30	27.9 ($r^2 = 0.88$)	12.3	—	—	2.5 (1300)
II	25	30	22.5 ($r^2 = 0.93$)	17.0	—	—	8.0 (1300)
II	20	30	19.2 ($r^2 = 0.91$)	19.0	—	—	15.0 (1300)
III	29	30	59.1 ($r^2 = 0.99$)	9.7	9.7	10.4	1.0 (1000)
III	25	30	52.0 ($r^2 = 0.89$)	13.4	10.3	13.7	2.0 (1000)
IV	29	30	40.5 ($r^2 = 0.79$)	11.0	9.6	11.3	1.0 (720)
IV	25	30	43.0 ($r^2 = 0.91$)	12.6	11.6	12.0	1.0 (720)
IV	20	30	29.4 ($r^2 = 0.89$)	18.6	15.4	18.2	4.0 (720)
V	25	29	39.1 ($r^2 = 0.96$)	—	—	—	18.0 (48)
V	25	25	27.1 ($r^2 = 0.97$)	—	—	—	16.0 (49)
V	25	19	15.4 ($r^2 = 0.91$)	—	—	—	17.5 (52)
VI	25	30	43.6 ($r^2 = 0.96$)	14.3	12.5	14.4	2.0 (620)
VI	25	25	35.1 ($r^2 = 0.94$)	17.6	13.3	14.6	2.0 (620)
VI	25	20	38.9 ($r^2 = 0.94$)	>22.0	14.0	15.2	1.0 (620)

In Experiments I and II, larvae were fed every other day, in all other experiments larvae were fed every day. Dashes indicate sampling from batch culture (Expts. I and II) or data not available (Expt. V).

the maximum shell length was measured with the larva lying on its left side. The presence of gill filaments and the lateral shell brims characterizing advanced larvae of this species (Pechenik and Lima, 1984) were also noted. Growth rates (μm shell growth $\cdot day^{-1}$) were determined by linear regression analysis of changes in shell length through time (SPSS Inc., 1988). The percentage of the larval population that was gilled or brimmed was plotted against time. From these plots we estimated the number of days necessary after larvae were released from their egg masses for 50% of the larvae in a population to become gilled or brimmed.

Table II

Influence of salinity on larval survival and rate of becoming competent in Experiment V

Salinity (PPT)	% Mortality	% population competent after 7 days in each salinity treatment $\bar{X} \pm$ SD (n)
29	18%	37% ± 4.0 (3)
25	16%	10.3% ± 9.3 (3)
19	17.5%	3.3% ± 2.9 (3)
14.5	31%	20% ± 7.2 (3)
8	85%	0% ± 0.0 (3)
4	100% in 3 days	—

At the time of KCl exposure, larvae were 16 days old. Larvae were introduced to the reduced salinities after 9 days of culture at full-strength salinity (29 ppt).

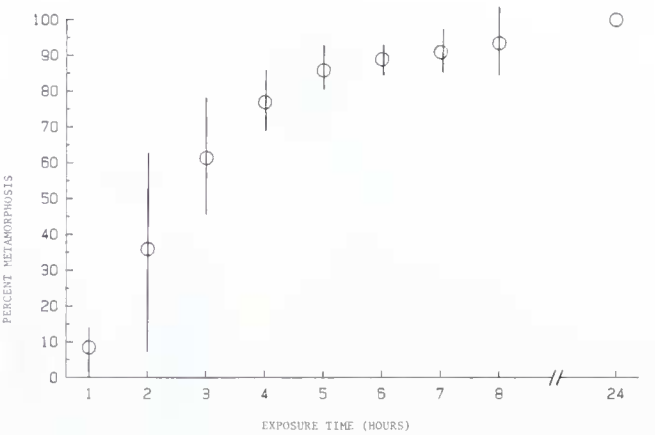

Figure 1. Influence of time exposed to elevated KCl concentration on metamorphosis of competent *Crepidula plana* larvae. KCl concentrations were elevated by 20 m*M* at 22–24°C. Larvae were examined for loss of velar lobes hourly for 8 h, then at 10 h and 24 h. Each point represents the mean of five replicates, with 20–21 larvae per replicate. Vertical bars represent one standard deviation. Average larval shell length (±SD) was 777 μm ± 97.8 (n = 100).

Larvae collected from batch culture were preserved in 10% formalin buffered with sodium borate (BORAX) (pH ~ 8.0), for later determination of larval organic weight; larvae reared at different temperatures were stored separately. Larval organic weights were determined as follows: one or more larvae of known shell lengths were placed into pre-weighed aluminum pans; the preserved animals were first rinsed three times with distilled water to remove preservatives and salts. The animals were dried overnight at 60°C in a drying oven, then weighed to determine initial total (inorganic and organic) dry weights. The animals were weighed to the nearest microgram (μg) with a Cahn microbalance with desiccant present in the weighing chamber to prevent rehydration. The pans were reweighed after sample combustion in a muffle furnace at 550°C for 6 h; combustion did not change the weight of the aluminum pans. The weight lost in combustion is equivalent to the larval organic weight. Individual body weights were

Table III

Results of ANACOVAs for shell size, % of the population competent to metamorphose, % fully gilled, or fully brimmed for each experiment by temperature and salinity with age as a covariate

Experiment I:	Influence of temperature (at 30 ppt) on growth rate	25°C > 20°C > 29°C
	Rate at which the population became competent	29°C > 25°C > 20°C
	Rate at which the population became gilled	29°C = 25°C = 20°C
	Rate at which the population became brimmed	29°C = 25°C = 20°C
Experiment II:	Influence of temperature (at 30 ppt) on growth rate	29°C > (25°C = 20°C)
	Rate at which the population became competent	29°C > 25°C > 20°C
	Rate at which the population became gilled	29°C = 25°C = 20°C
	Rate at which the population became brimmed	29°C = 25°C = 20°C
Experiment III:	Influence of temperature (at 30 ppt) on growth rate	29°C > 25°C
	Rate at which the population became competent	29°C > 25°C
	Rate at which the population became gilled	29°C > 25°C
	Rate at which the population became brimmed	29°C > 25°C
Experiment IV:	Influence of temperature (at 30 ppt) on growth rate	25°C > 29°C > 20°C
	Rate at which the population became competent	29°C > 25°C > 20°C
	Rate at which the population became gilled	29°C > 25°C > 20°C
	Rate at which the population became brimmed	29°C > 25°C > 20°C
Experiment VI:	Influence of salinity (at 25°C) on growth rate	30 ppt > 20 ppt > 25 ppt
	Rate at which the population became competent	30 ppt > 25 ppt > 20 ppt
	Rate at which the population became gilled	30 ppt > (25 ppt = 20 ppt)
	Rate at which the population became brimmed	30 ppt > 25 ppt > 20 ppt

All differences are significant at $P < 0.05$, and most were significant at $P < 0.001$.

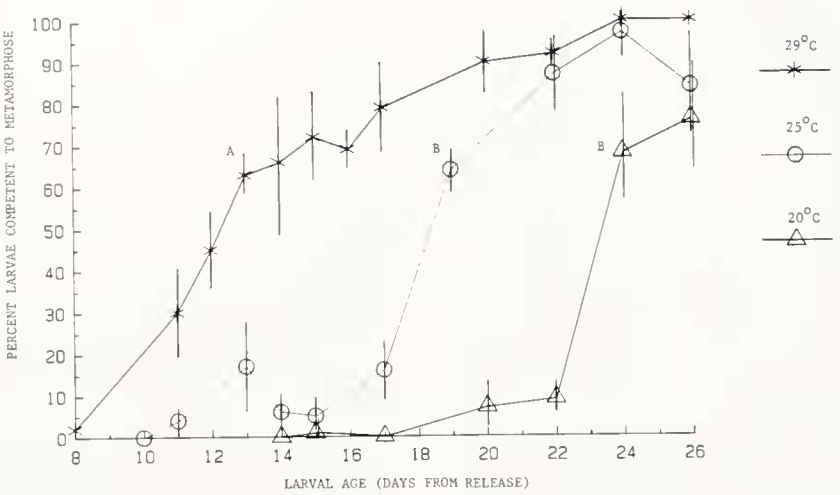

Figure 2. Influence of rearing temperature on the rate at which larvae became competent to metamorphose in Experiment 1. Each point represents the mean percentage metamorphosing in three bowls, with 34–40 larvae per bowl. Vertical bars represent one SD about the mean. Different letters represent larval populations with different mean growth rates (A < B).

determined for larvae longer than 700 μm; larvae less than 700 μm were pooled for weight determinations.

Determining the effect of temperature on the rate of becoming competent to metamorphose

Pechenik and Heyman (1987) found that elevating the KCl levels in natural seawater by 20 m*M* induced competent larvae of *C. fornicata* to metamorphose within 7 h. To determine whether the larvae of *C. plana* would respond similarly, we exposed advanced larvae of this species (22-day-old, 770 ± 98 μm shell length, n = 100) to a 20 m*M* increase in KCl concentration. We checked hourly for larval metamorphosis for the first 8 h, then at 10 h and 24 h; newly metamorphosed larvae were removed at each observation. The experiment was conducted at 22°C, with 5 replicates (21 larvae per replicate).

To determine the effect of temperature on the rate at which larvae in a given population became competent to metamorphose, we monitored larvae from a temperature treatment until some individuals reached shell lengths of about 600 μm. At 1–3 day intervals, we then transferred all larvae from three randomly chosen bowls into 3 bowls of seawater with elevated KCl concentrations; 30 to 45 glass bowls of larvae (20–40 larvae per bowl, depending on the experiment) were used for each temperature treatment during the course of an experiment. After exposing larvae to the elevated KCl for 6 h, we determined the number of individuals that had metamorphosed in each bowl, and measured the shell lengths of those that had metamorphosed and of those that had not. We also determined whether individuals had gills or shell brims. We conducted *t*-tests to determine whether there were differences in the mean shell lengths of competent and precompetent larvae in each temperature treatment. The rate at which larvae in each population became competent was determined by linear regression analysis. Significant regression coefficients (r) were obtained in all experiments. For regressions with correlation coefficients (r^2) greater than 0.80, the number of days for 50% of the larval population to become competent was determined from the regression. For data with r^2 values less than 0.80, the number of days for the populations to become 50% competent was estimated by eye.

Determining the influence of temperature on maximum length of larval life

Larvae of *C. plana* eventually undergo "spontaneous" metamorphosis in the laboratory, even when maintained in frequently cleaned glassware (Lima and Pechenik, 1985). Three bowls (20–40 larvae per bowl, depending on experiment) at each temperature were washed and acid-rinsed daily, at each change of algal suspension. Larvae were examined daily; we counted, removed, and measured newly metamorphosed snails. These data were compared with observations on the mean age and size, at metamorphosis, of individuals cultured in bowls cleaned only every 48 h ("filmed bowls"). The aim was to determine whether biological films building up over the 48-h period would induce a greater number of larvae to metamorphose. Such biological surface films have been implicated as metamorphic inducers in many marine invertebrates (Meadows and Campbell, 1972; Scheltema, 1974; Kirchman *et al.*, 1982; Lima, 1983; Coon *et al.*, 1985; Weiner *et al.*, 1989).

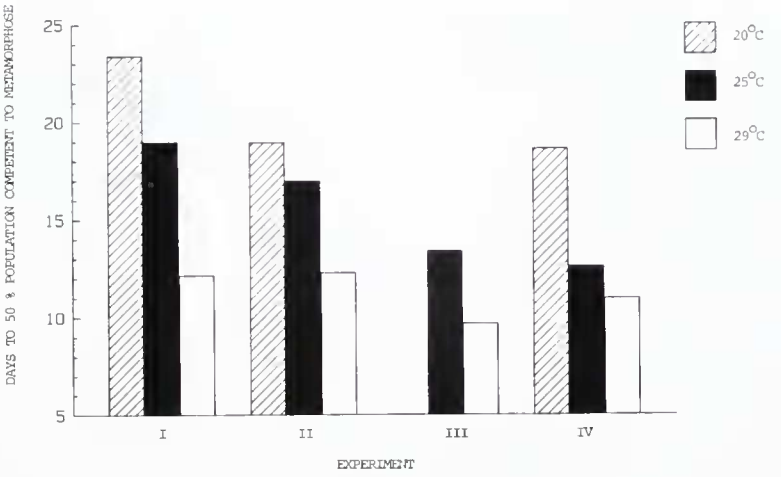

Figure 3. Influence of rearing temperature on the number of days for 50% of the larvae in each treatment population to become competent to metamorphose in Experiments I–IV.

The time required for 50% of the population to metamorphose in the bowls cleaned daily, minus the time required for 50% of the larvae to become competent in parallel experiments, was used as an index of capacity for delaying metamorphosis. This cannot be used to predict dispersal potential in the field, but should enable us to assess the influence of temperature and salinity on the physiological capacity for prolonging larval life, and will permit future interspecific comparisons of the physiological capacity for delaying metamorphosis.

Determining the effects of salinity on rates of growth, morphological differentiation, and rates of becoming competent

In two experiments, we examined how salinity affected the relationship between rates of growth, morphological differentiation, and becoming competent. In the first experiment, six salinities [29, 25, 19, 14.5, 8, and 4 parts per thousand (ppt)] were used to determine the salinity tolerance of larval *C. plana;* these salinities are equivalent to osmotic concentrations of 821, 708, 557, 403, 223, and 116 mOsm, respectively. The five lowest salinities were made by mixing 0.45 μm filtered seawater with deionized water; the 29 ppt seawater was composed solely of undiluted 0.45 μm filtered seawater. Osmotic concentrations were measured with a freezing point depression osmometer (Advanced Instruments, Inc.). This experiment was conducted at 25°C, with three replicate bowls of 20 larvae per bowl in each salinity treatment. Water and food were replaced daily. All larvae were reared in full-strength seawater for 9 days, and then acclimated to lower salinities in stages during 1 h. Shell-less, moribund, or dead larvae were counted and removed daily. Shell lengths were measured non-destructively (Pechenik, 1984) each day for growth rate determinations. All larvae were exposed to an increase of 20 mM KCl on the seventh day of the experiment (the 16th day of larval life) to determine the percentage of larvae competent to metamorphose in each salinity.

Based on the results of the first experiment, a second experiment (Experiment VI) was conducted at 30, 25, 20 ppt (again at 25°C) to examine more fully the effect of salinity on rates of growth and differentiation. We reared 25 larvae per bowl with 31 bowls per treatment. To minimize the effects of food supply on salinity—algae are cultured at about 30 ppt—the algae were concentrated by centrifugation at 3000 $\times$ g for 12 min and then resuspended in seawater of the appropriate test salinity (Pechenik and Fisher, 1979). Algal cells remained alive and motile in all salinities. Every day, larval shell lengths were measured non-destructively from randomly selected bowls at each salinity; presence or absence of gill filaments and shell brims were simultaneously noted.

Periodically, three bowls of larvae from each salinity treatment were randomly selected and all individuals (20–30 larvae per bowl) were exposed to elevated KCl concentrations in seawater to assess metamorphic competence. Larvae reared at 30 or 25 ppt were exposed to an increase of 20 mM KCl while those reared in 20 ppt seawater were exposed to either a 20 or a 23 mM KCl increase, to compensate for the lower baseline KCl concentration at the reduced salinity. All individuals exposed to KCl were measured, whether or not they metamorphosed, and were examined for the presence of gill filaments and shell brims.

Statistical analyses

Analyses of covariance (ANACOVA) were conducted for each experiment. Either temperature or salinity were

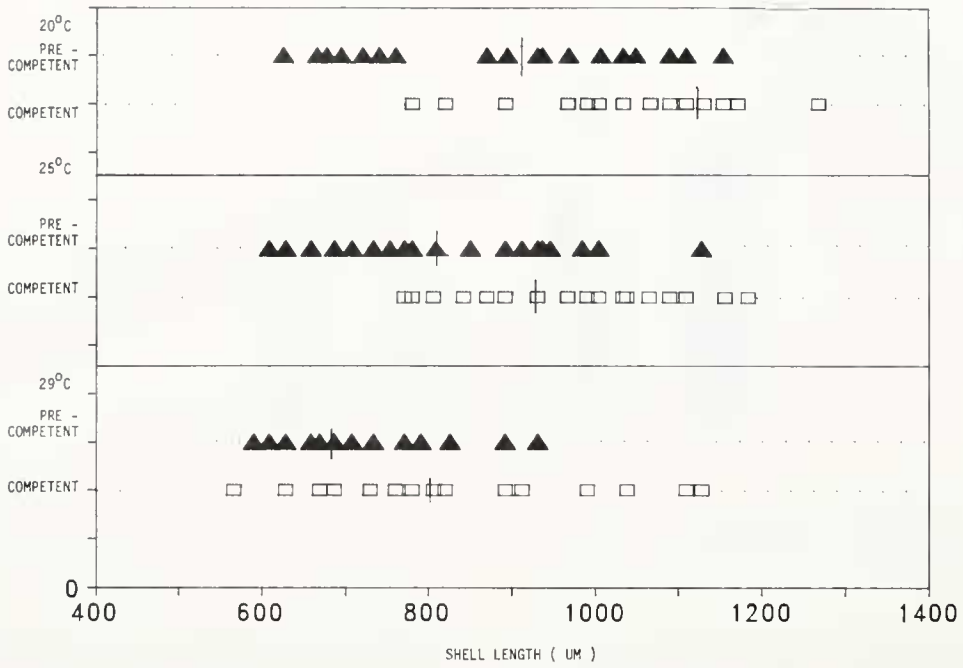

Figure 4. A comparison of the shell lengths of competent (□) and pre-competent (▲) larvae of *Crepidula plana* from Experiment IV. The points within each treatment represent the response of larvae from three bowls (~60 larvae per bowl). Data were taken when larvae at 29°C were 11 days old ($\bar{x}$ = 50.0% larvae competent; SD = 4.3); larvae at 25°C were 13 days old ($\bar{x}$ = 54.6% competent; SD = 6.4); larvae at 20°C were 19 days old ($\bar{x}$ = 51.3% competent; SD = 14.1).

used as independent variables; age (days from hatch) was the covariate; and one of the following was taken as the dependent variable: percent of the larval population competent to metamorphose, percent of the larval population gilled, percent of the larval population with a complete shell brim, or shell length (Table I) (Kleinbaum *et al.*, 1988; SPSS, Inc. 1988). In Experiments I and II, the gill, shell brim, and shell length data were obtained from larvae in batch culture, whereas the rate at which larvae became competent to metamorphose was determined with larvae reared in glass bowls. In Experiments III–VI, all data were obtained from the larvae reared in glass bowls. Percentage data were arcsine transformed prior to subsequent analysis, using the formula for proportions with unequal sample sizes (Draper and Smith, 1981).

Results

Effects of temperature and salinity on survival

Larval survivorship was high at all temperatures in Experiments I–IV, with the best survival, greater than 96%, occurring at the highest temperature tested (29°C) (Table I).

However, larvae were intolerant of very low salinities (Table II). Within the first two hours at 4 and 8 ppt, larvae were found clumped together with mucus, mainly on the bottoms of the rearing bowls, with their velar lobes extended and velar cilia moving; all treatment bowls at higher salinities (14.5, 19, 25, 29 ppt) contained swimming larvae. On the second day, at 4 and 8 ppt, velar lobes appeared smaller and velar cilia were less visible. By the third day, all larvae in the 4 ppt seawater had died and only two larvae out of the initial 65 survived at 8 ppt. Larval survivorship was good at salinities of 19 ppt and above, particularly in the second salinity experiment (Table I, Experiment VI).

Effects of temperature on rates of growth and morphological differentiation

Temperature had no significant effect on size-specific organic weight at 20 and 25°C and at 20 and 29°C (*t*-tests between slopes, $P > 0.10$, $t = 0.69$, d.f. = 30 and $t = 0.26$, d.f. = 42, respectively). Thus, a given change in shell length reflected comparable growth (in organic weight) for larvae at 20 and 25°C, and at 20 and 29°C. However, a given change in shell length reflected greater growth (in organic weight) for larvae at 25°C as compared to larvae reared at 29°C (*t*-tests, $P < 0.05$, $t = 2.05$, d.f. = 50).

The effect of temperature on larval growth rate varied markedly among experiments (Experiments I–IV, Tables I and III). There were differences both in the average

Table IV

Influence of temperature on age and size at spontaneous metamorphosis in glassware cleaned daily (clean bowls) and the delay period (number of days between when 50% of the population was competent and the mean age at metamorphosis in clean bowls)

Experiment number	Temperature (°C)	Mean age (days) at spontaneous metamorphosis (clean) $\bar{X} \pm SD$ (n)	Delay period (days)
I	29	19.32 ± 4.9 (75) A	7.16
I	25	24.66 ± 3.8 (98) B	5.67
I	20	28.66 ± 3.8 (100) C	5.26
II	29	18.09 ± 5.4 (99) A	5.79
II	25	26.86 ± 4.9 (44) B	9.86
II	20	30.96 ± 3.2 (73) C	12.00
III	29	14.49 ± 2.2 (306) A	4.79
III	25	16.81 ± 1.6 (214) B	3.40
IV	29	16.79 ± 1.7 (24) A	5.79
IV	25	18.54 ± 1.4 (24) B	5.90
IV	20	24.85 ± 2.6 (39) C	6.21

Within each column, letters following sample sizes signify significantly ($P < 0.05$) different means within experiments.

amount of daily growth at a temperature and in how temperature affected relative growth rates. For example, larvae grew the slowest (28 $\mu m \cdot day^{-1}$) at 29°C in Experiment I, but grew the fastest at 29°C (28 $\mu m \cdot day^{-1}$) in Experiment II (Table I). Over all experiments, average growth rates ranged between 19 $\mu m \cdot day^{-1}$ (Experiment II, 20°C) and 59 $\mu m \cdot day^{-1}$ (Experiment III, 29°C).

Larvae generally developed gill filaments and shell brims more rapidly at higher rearing temperatures, although rates of gill and brim formation were independent of temperature in the first two experiments (Table III). Note that in Experiment IV the effects of temperature on growth rates did not parallel those on morphological differentiation rates. These data indicate that gill formation, brim formation, and growth rate were affected similarly by rearing temperature in only one of the four experiments (Experiment III); only two temperature treatments were tested in that experiment.

Larvae typically became gilled between about 620–820 μm and brimmed between about 710–850 μm, with no consistent influence of rearing temperature or feeding frequency. Some larvae within the populations were fully gilled and brimmed before other larvae in the same population became gilled, indicating much individual variation in rates of morphological development within each temperature treatment.

All but seven metamorphosed individuals—out of thousands of metamorphosed snails examined in these experiments—had conspicuous gills, suggesting that most larvae developed gills before they became competent to metamorphose. The seven gill-less juveniles were all found at 29°C (Experiments I and II).

Elevated KCl concentration stimulates metamorphosis

Response to elevated KCl was rapid. Of those 22-day-old individuals (22°C) that eventually responded, increasing KCl concentrations by 20 m*M* induced at least 90% to metamorphose within 6 h (Fig. 1). Thus, in all subsequent experiments, larvae were exposed to elevated KCl concentrations for 6 h to assess metamorphic competence, defined here by the response to elevated potassium.

Effect of temperature on rates of becoming competent to metamorphose

Despite the unpredictable effects of rearing temperature on rates of growth and morphological differentiation, increasing larval rearing temperature significantly increased ($P < 0.001$) the rates at which larvae became competent to metamorphose in all experiments (Tables I, III; Figs. 2, 3).

Larval shell length was a poor indicator of whether a larva was competent to metamorphose. Although competent larvae were, on average, significantly larger ($P < 0.0001$) than pre-competent larvae of the same age and rearing history, shell lengths of competent and pre-competent larvae overlapped in all experiments, as exemplified by Experiment IV (Fig. 4).

Effect of temperature on the maximum length of larval life and period of delayed metamorphosis

At higher temperatures, larvae consistently exhibited "spontaneous" metamorphosis sooner than at lower temperatures (Table IV and Fig. 5). However, average growth rates failed to predict rates of spontaneous metamorphosis within a population. In Experiment I, for example, larvae reared at 20°C or 25°C grew at equivalent rates but metamorphosed faster at the higher temperature (Fig. 5). Even

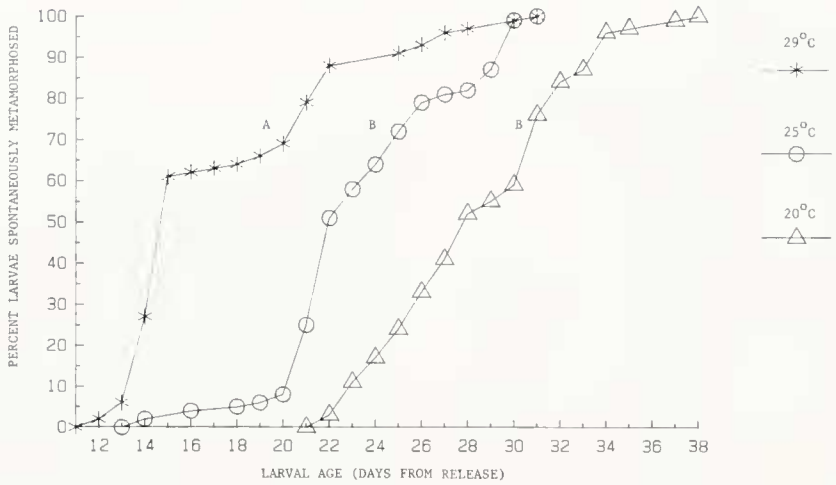

Figure 5. Maximum length of larval life for *Crepidula plana* maintained in glass bowls, acid-washed daily (Experiment I). Each point represents the mean of three replicates (110 larvae per treatment). Different letters signify larval populations differing significantly in mean growth rates (A < B < C).

so, individuals exhibiting faster growth within a temperature treatment tended to metamorphose sooner than slower growing larvae reared at the same temperature, confirming previous results (Lima and Pechenik, 1985) (Fig. 6; regression analysis of log growth rate). Within each temperature, faster growing individuals also tended to metamorphose at larger shell lengths, although the data do show considerable scatter (Fig. 7; $P < 0.05$ at each temperature). Individual growth rates were estimated using age and size at metamorphosis (Lima and Pechenik, 1985).

Generally, larvae maintained in bowls cleaned only every 48 h metamorphosed significantly sooner ($P < 0.05$; t-test), by about 5–10 days, than larvae maintained in bowls cleaned every 24 h, and at smaller shell lengths [smaller by about 100–300 μm (Zimmerman, 1989)]. This indicates that microbial films formed over 48 h could trigger larvae of *C. plana* to metamorphose, supporting previous reports (Lima, 1983).

The average delay period, defined here by the difference (in days) between (a) mean age at "spontaneous" metamorphosis in bowls cleaned daily and (b) when 50% of a larval population was competent to metamorphose, varied between experiments, and was markedly altered by temperature only in Experiment II (Table IV).

Effect of salinity on rates of growth and morphological differentiation

The effects of salinity on growth rate differed in the two experiments. In Experiment V (Table I), larvae grew more quickly at higher salinities (by about 12 $\mu m \cdot day^{-1}$ for each salinity increase above 19 ppt). In the three lowest salinities (4, 8, and 14.5 ppt), larvae suffered high mortality (85–100% at 4 and 8 ppt) and exhibited no detectable growth. In Experiment VI, salinity significantly affected mean growth rates, but not as dramatically as in Experiment V, and not in direct proportion to salinity. Larvae reared at 20 ppt grew significantly faster than larvae at 25 ppt in Experiment VI (Tables I, III). In both salinity experiments, larvae reared in full strength seawater (either 29 or 30 ppt) grew at rates comparable to those of larvae reared under comparable conditions (25°C, full strength seawater) in Experiments I–IV (Table I).

Salinity over the range of 20–30 ppt had negligible effects on rates of gill formation (Tables I, V) and on the shell sizes at which larvae became either gilled or brimmed. Larvae became gilled and brimmed at shell sizes between 628–728 μm and 699–790 μm, respectively, regardless of rearing salinity. However, every increase in rearing salinity increased rates of shell brim formation (Table III). The pattern of significant salinity effects on rates of growth and on rates of gill and brim formation (Table III) indicates that rates of growth and morphological differentiation were not affected similarly by changes in salinity.

The relative effect of salinity on rates of becoming competent to metamorphose and rates of growth

Despite the erratic influence of salinity on rates of growth, gill, and shell brim formation, larvae reared at higher salinities typically became competent to metamorphose sooner than those reared at lower salinities in both Experiments V and VI (Tables I–III). These results suggest that changes in salinity may uncouple rates of growth, rates of morphological differentiation, and rates of becoming competent to metamorphose. In Experiment

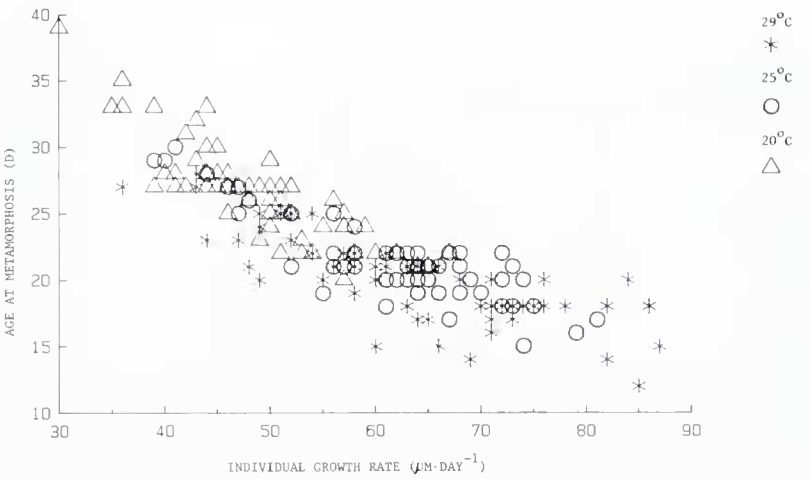

Figure 6. Maximum length of larval life as a function of estimated individual growth rate ($\mu m \cdot day^{-1}$) in Experiment IV ($r^2 = 0.74$, $y = -16.4(\ln x) + 88.7$). Individual growth rates were estimated from the size and age at which each individual underwent spontaneous metamorphosis in glass bowls that were cleaned daily. Larvae were cultured at three temperatures, as indicated (n = 64, 62, and 63 larvae per treatment at 29°C, 25°C and 20°C, respectively).

VI, for example, larvae grew more rapidly at 20 ppt than at 25 ppt, but took longer to become competent at the lower salinity (Table III and Fig. 8). Experiment VI was terminated before all larvae were allowed to metamorphose, so calculation of age and size at metamorphosis was not possible.

There was no significant difference ($P > 0.05$) in the percentage of larvae induced to metamorphose when KCl concentrations were elevated by 20 *versus* 23 m*M* at 20 ppt. Thus, the dilution of full strength seawater to make 20 ppt and 25 ppt seawater did not significantly affect the ability of KCl to induce larval metamorphosis.

Discussion

The primary goal of these experiments was to determine, for *Crepidula plana*, whether changes in temperature and salinity alter rates of growth, morphological differentiation, and the onset of competence equally. We must first consider the effects of temperature and salinity on each of these three components of development individually.

Larvae grew significantly faster at progressively higher temperatures (Table I) in only one experiment (Experiment III). Lima and Pechenik (1985) also found an in-

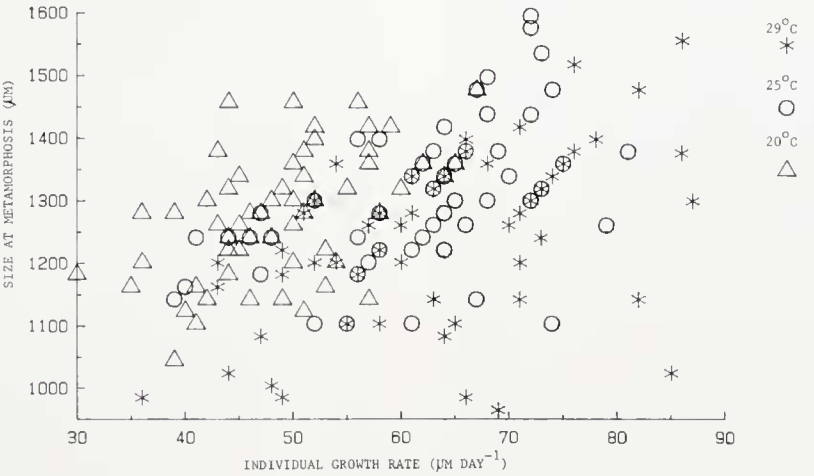

Figure 7. Size at metamorphosis as a function of individual larval growth rate ($\mu m \cdot day^{-1}$) (Experiment IV). Faster growing larvae tended to spontaneously metamorphose at larger shell sizes ($P < 0.05$; r = 0.549 at 29°C, n = 64; r = 0.512 at 25°C, n = 62; r = 0.511 at 20°C n = 63; combined r = 0.249, n = 189). Growth rates were estimated from size and age at spontaneous metamorphosis. Larvae were reared at three temperatures, as indicated.

Table V

Influence of temperature and salinity on percent changes in rates of shell growth, morphological differentiation, becoming competent to metamorphose, and spontaneous metamorphosis for all experiments

Experiment number	Temperature, salinity (°C) (ppt)	Growth rate ($\mu m \cdot day^{-1}$)	1·(Time to 50% competent)$^{-1}$	1·(Time to 50% gilled)$^{-1}$	1·(Time to 50% brimmed)$^{-1}$	1·(Time to 50% spontaneously metamorphosed)$^{-1}$
I	20°C, 30 ppt	—	—	—	—	—
	25°C, 30 ppt	+18%	+19%	—	—	+21%
	29°C, 30 ppt	−16%	+48%	—	—	+48%
II	20°C, 30 ppt	—	—	—	—	—
	25°C, 30 ppt	+17%	+10%	—	—	+11%
	29°C, 30 ppt	+45%	+35%	—	—	+48%
III	25°C, 30 ppt	—	—	—	—	—
	29°C, 30 ppt	+14%	+28%	+6%	+24%	+18%
IV	20°C, 30 ppt	—	—	—	—	—
	25°C, 30 ppt	+46%	+32%	+25%	+34%	+22%
	29°C, 30 ppt	+38%	+41%	+38%	+38%	+29%
V	25°C, 8 ppt	—	—	—	—	—
	25°C, 14.5 ppt	+12%	+20%	—	—	—
	25°C, 19 ppt	+233%	+3.3%	—	—	—
	25°C, 25 ppt	+334%	+10%	—	—	—
	25°C, 29 ppt	+438%	+37%	—	—	—
VI	25°C, 20 ppt	—	—	—	—	—
	25°C, 25 ppt	−9%	+20%	+5%	+4%	—
	25°C, 30 ppt	+12%	+35%	+11%	+6%	—

Each percent change was calculated relative to the lowest temperature, salinity treatment in a particular experiment.

consistent effect of temperature on larval growth rate for larvae of *C. plana* fed *T-ISO*, and the larval growth rates reported here for *C. plana* are generally comparable to those previously reported by Lima and Pechenik (1985) for larvae reared at identical food concentrations and temperatures. In our Experiment III, however, larvae reared at 29°C and 25°C grew 1.5–2.0 times faster than those reared by Lima and Pechenik (1985) under the same conditions. Lima and Pechenik (1985) reported comparably high larval growth rates (exceeding 50 $\mu m \cdot day^{-1}$) for *C. plana* reared at 25°C and 29°C on a different naked flagellate, *Isochrysis galbana* (clone ISO). Larvae of the congener *C. fornicata* also grow at rates exceeding 50 $\mu m \cdot day^{-1}$, at temperatures above 24°C (Lucas and Costlow, 1979; Pechenik, 1984; Pechenik and Lima, 1984).

Salinity also influenced larval growth rates, although the effects were often inconsistent between the two experiments (Tables I, III). Larvae grew faster at progressively higher salinities in Experiment V, but not in Experiment VI (Tables I, III). As with other molluscan larvae, including the congener *C. fornicata* (Davis, 1958; Davis and Ansell, 1962; Davis and Calabrese, 1964; Scheltema, 1965; Calabrese and Rhodes, 1974; Robert *et al.*, 1988; His *et al.*, 1989), those of *C. plana* grew poorly at salinities below about 20 ppt.

The influence of temperature on rates of morphological differentiation also varied from one experiment to the next (Table III). This contrasts with results reported for *C. fornicata* by Pechenik and Lima (1984) and Pechenik (1984), who found that larvae always tended to develop gills and shell brims more rapidly at higher temperatures. We have no way of knowing whether the inter-experiment variation we report for *C. plana* reflects genetic differences in the larval populations used, subtle differences in rearing conditions among experiments, or differences in the physiological history of the adults that released the larvae used in these experiments (Bayne *et al.*, 1975). Increases in salinity did not predictably alter rates of gill formation in Experiment VI (Table III), but shell brims formed more rapidly at higher salinities.

As reported previously for larvae of *C. plana* and *C. fornicata* (Pechenik and Lima, 1984; Lima and Pechenik, 1985), and for larvae of the blue mussel *M. edulis* (Pechenik *et al.*, 1990), temperature apparently altered rates of growth and morphological development to different degrees. In our studies, this is suggested by the fact that larvae tended to develop shell brims and visible gill filaments at different sizes when reared at different temperatures. For example, in Experiment I, larvae formed visible gill filaments on average between 681 and 721 μm,

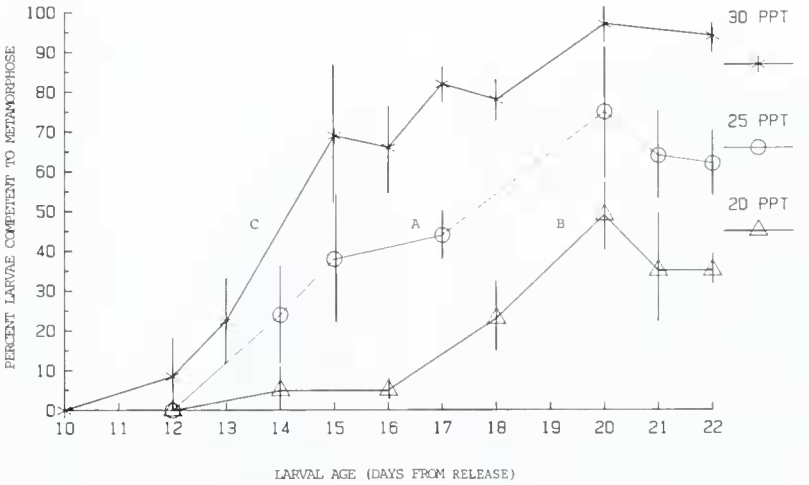

Figure 8. Influence of salinity on the rate at which larvae became competent to metamorphose in Experiment VI. Each point represents the mean percentage of larvae competent in three bowls, with 20–23 larvae tested per bowl. Vertical bars represent one SD about the mean. Different letters represent larval populations with different mean growth rates (A < B < C).

between 675 and 817 μm, and between 742 and 786 μm, at 29°C, 25°C, and 20°C, respectively. Rates of shell growth would have to be altered by temperature in exact proportion to any changes in rates of morphological development if larvae are to form gills and shell brims at comparable average sizes in all rearing conditions (Pechenik and Lima, 1984; Pechenik *et al.*, 1990).

Rates of shell growth and morphological differentiation were also affected to different degrees by salinity. For example, in Experiment VI, larvae grew significantly faster at 20 ppt than at 25 ppt, but the salinity decrease did not affect rate of gill formation. Indeed, rate of gill formation was not affected by salinity over the range tested. In contrast, shell brims formed faster at the higher salinity.

Despite the generally unpredictable effects of temperature on rates of larval growth and morphological differentiation both among and, often, within experiments, the influence of temperature on the rates at which larvae became competent to metamorphose was remarkably consistent among all four experiments; larvae always became competent to metamorphose faster when reared at higher temperatures (Table III and Fig. 3). Rates of becoming competent to metamorphose were clearly uncoupled from rates of morphological differentiation and shell growth. In Experiment I, for example, larvae reared at 29°C became competent significantly sooner (and often at smaller sizes) than larvae reared at 20°C or 25°C, despite significantly slower average growth for larvae reared at the higher temperature (Figs. 2, 4). In addition, larvae reared at 25°C became competent significantly sooner than larvae at 20°C, even though these larvae did not grow at significantly different rates at the two temperatures.

The same was true of the experiments (V and VI) examining the influence of salinity. Here again, larvae reared at higher salinities generally became competent faster, while rates of growth and morphological differentiation were not so predictably affected. For example, in Experiment VI, larvae reared at 20 ppt grew significantly faster than larvae at 25 ppt, but those larvae reared at 20 ppt became competent at slower rates (Table III and Fig. 8). The influence of temperature or salinity on the amount of time required for larvae in a population to become competent clearly cannot be predicted from the effects of environmental change on rates of growth (Table V). Individual competence also cannot be predicted on the basis of shell length (Fig. 4) or the presence of a shell brim or visible gill filaments; at least some gill-less larvae were induced to metamorphose by elevating KCl concentration (at 29°C, Experiments I and II). Also, in every experiment, at every temperature, some larvae without shell brims could be induced to metamorphose. Similarly, neither shell size nor morphological indicators were adequate predictors of whether individual blue mussel larvae would or would not attach to filamentous substrates in the laboratory (Eyster and Pechenik, 1987), or when oyster larvae (*Crassostrea gigas*) would exhibit settlement behavior in response to L-DOPA (Coon *et al.*, 1990).

Variation in the rates at which individuals became competent to metamorphose within treatments (as in Figs. 2 and 8) may be a natural phenomenon that encourages larvae released from an individual female to metamorphose at different times, likely increasing the spread of siblings among different populations (Strathmann, 1974; Hadfield, 1977) and minimizing their competition for food and space as juveniles.

In our experiments, larvae consistently underwent

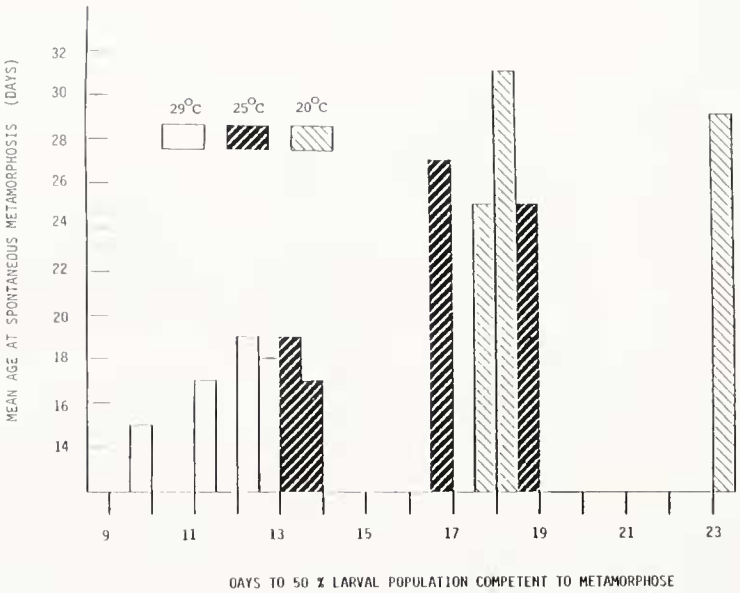

Figure 9. Influence of temperature on the relationship between the maximum length of larval life and the time required for 50% of a population to become competent. Larvae of *Crepidula plana* were reared at three temperatures, as indicated. For each temperature, different bars represent data from different experiments.

"spontaneous" metamorphosis sooner at higher temperatures (Fig. 5 and Table IV). This phenomenon of molluscan larvae metamorphosing sooner in warmer temperatures has been noted previously (Loosanoff, 1959; Davis and Ansell, 1962; Davis and Calabrese, 1964; Bayne, 1965; Pechenik, 1984; Pechenik and Lima, 1984; Lima and Pechenik, 1985). This relationship is consistent with the hypothesis that the timing of spontaneous metamorphosis is determined by the rate at which larvae progress through a developmental program with a fixed endpoint (Pechenik, 1980, 1984; Pechenik and Lima, 1984); the endpoint could be determined by some endogenous controlling factor or, as suggested recently by Coon *et al.* (1990), could reflect a gradually increasing sensitivity of receptors for an external chemical cue present naturally in extremely low concentrations. Although mean growth rates were not adequate indicators of the rates at which larvae within a population would undergo spontaneous metamorphosis (Fig. 5), faster growing individuals did tend to exhibit spontaneous metamorphosis sooner than slower growing individuals (Fig. 6). These results are similar to those reported previously for this species (Lima and Pechenik, 1985), for the congener *C. fornicata* (Pechenik, 1984; Pechenik and Lima, 1984), and for the bivalves *Mercenaria mercenaria* (Loosanoff, 1959) and *M. edulis* (Beaumont and Budd, 1982).

Despite the pronounced influence of temperature on rates of becoming competent and rates of spontaneous metamorphosis (Tables III, IV), temperature had a minor influence on the length of time that larvae of *C. plana* delayed metamorphosis in frequently cleaned glass bowls in all but one experiment (Table IV and Fig. 9). Only in Experiment II did temperature affect delay period by more than one or two days (Table IV). Thus, although larvae of *C. plana* will have a longer pre-competent period at lower temperatures, the capacity for delaying metamorphosis in the absence of suitable substrate may be affected to a much lesser degree.

In our experiments, larvae metamorphosed "spontaneously" about 3.5–12 days after becoming competent, with most delay periods lying between about 5 and 7 days (Table IV). These data are comparable to earlier laboratory estimates of delay potential for larvae of this species reared at comparable temperatures (Lima and Pechenik, 1985: their Table II), and seem to confirm the reduced capacity of *C. plana* for postponing metamorphosis relative to that exhibited by larvae of *C. fornicata;* the maximum delay period of about 20–30 days suggested for *C. fornicata* (Pechenik, 1984) is only an estimate, however, and has not yet been confirmed. The estimates of delay potential for *C. plana* reared at different temperatures given by Lima and Pechenik (1985) were based on the assumption that competence is attained at a particular shell length. The good agreement between their estimates and our more direct determinations suggest that although individual larvae clearly do not become competent to metamorphose at a particular size, the simplifying assumption of length-related competence may permit adequate predictions at the population level.

Clearly, the various aspects of morphological and

physiological development are affected to different degrees in *C. plana* by temperature and salinity changes. In only one experiment (Experiment III) did increased rearing temperature significantly increase all components of developmental rate that were monitored: shell and tissue growth, timing of gill differentiation and shell brim development, and onset of metamorphic competence. The likely impact of environmental factors on larval dispersal periods therefore cannot be estimated from data on rates of growth or morphological development, but clearly must be determined directly. Our data suggest that changes in temperature and salinity will have a more consistent influence on duration of pre-competent and competent periods of development than on either rates of shell growth or rates of morphological differentiation.

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